

REMARKS

Upon entry of the amendments to the claims herein, claims 1-12 are pending. Claims 3, 7 and 8 are amended. Claims 7 and 8 are amended for clarification purposes only. Support for the amendments to claim 3 can be found throughout the specification as originally filed including, *inter alia*, the following: Abstract and page 2, lines 12-23. Applicants submit that no new matter is introduced into the specification by way of the present amendments pursuant to 35 U.S.C. § 132. Applicants respectfully request entry of the amendments, reconsideration of the rejections, and allowance of the pending claims.

Claims 1, 2, and 4-6 have been withdrawn as being drawn to non-elected subject matter.

35 U.S.C. §112, second paragraph

Claims 7 and 8 are rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite. Applicants request reconsideration of these rejections in view of the present claim amendments.

35 U.S.C. § 103

Claims 3, 7, 9, 10, and 12 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Stamler (WO 96/30006). According to the Examiner, Stamler '006 teaches the concurrent administration of a hemoglobin-containing blood substitute and sodium nitrite, and also teaches the nitrosylation of proteins *in vivo* as a therapeutic modality for treating or preventing vasoconstriction associated with the infusion of blood substitutes containing hemoglobin.

Amended claim 3 recites a method of blood product transfusion comprising the steps of co-administering a deoxygenated hemoglobin-containing blood substitute and an inorganic nitrite into a patient via infusion, wherein the inorganic nitrite is infused at a rate of 0.01 to 10 micromoles per minute, and the hemoglobin-containing blood substitute is infused at a rate of 1 to 1000 cubic centimeters per hour.

The problem to be solved by the instant invention is how to provide a stable blood substitute for use in transfusions that does not cause constricting of the blood vessels. While S-

nitrosyl hemoglobin (SNO-Hb) is known to have vasodilator activity, it is not useful in blood substitutes or transfusions because it is unstable and thus cannot be stored without deteriorating (see Specification at page 1, lines 15-18). The present invention solves this problem by providing a method for preparing iron nitrosyl hemoglobin which is very stable and can be readily converted into SNO-Hb with vasodilator and anti-platelet activity upon *in vivo* administration (see Specification at page 1, line 21 to page 2, line 5). As stated in the specification as-filed, the present invention is based upon the discovery that low (physiological) concentrations of nitrite *do not* oxidize hemoglobin as previously thought, but instead combine with *deoxygenated* hemoglobin to store NO on heme β -subunit of hemoglobin tetramer to form iron nitrosyl hemoglobin. Upon oxygenation *in vivo*, the NO is transferred from the heme of β -subunits to thiol of β -cys93 to produce SNO-Hb (see Abstract and page 2, lines 12-23 of Specification).

Applicants submit that Stamler '006 fails to teach or suggest that an inorganic nitrite can be used in the disclosed method. Specifically, Stamler '006 fails to teach or suggest co-administering deoxygenated hemoglobin containing blood substitute and inorganic nitrite into the patient via infusion, wherein the inorganic nitrite is infused at a rate of 0.01 to 10 micromoles per minute, and the deoxygenated hemoglobin containing blood substitute is infused at a rate of 1 to 1000 cubic centimeters per hour.

The present invention also provides unexpected results which are not taught or suggested by Stamler '006. The Examiner asserts that Example 19 of Stamler '006 teaches that equimolar quantities of sodium nitrite and hemoglobin should be reacted and that the quantity of sodium nitrite and the rate of administration can be calculated from the quantity of hemoglobin to be administered. The Examiner further asserts that the claimed concentration range of inorganic nitrite (*i.e.*, infusion of inorganic nitrite at a rate of 0.01 to 10 micromoles per minute) cannot provide patentable subject matter unless there is a surprising result indicating that the concentration is critical.

The claimed infusion rate is in fact essential and critical to the claimed invention. It is well recognized in the art that hemoglobin is easily oxidized by inorganic nitrite resulting into methemoglobin, which can be toxic at elevated levels in the circulation. Co-infusion of an inorganic nitrite and a blood substitute containing hemoglobin at the claimed infusion rate of

0.01 micromoles to 10 micromoles per minute prevents toxic oxidization of the hemoglobin during infusion. Moreover, the instant invention does not require reacting equimolar quantities of sodium nitrite and hemoglobin (which would not be compatible with life), as taught in Stamler '006. As stated in the specification, the present invention is based upon the discovery that low concentrations of nitrite do not oxidize oxyhemoglobin, as thought, but instead combine with deoxygenated hemoglobin to store NO on heme β -subunit of hemoglobin tetramer to form iron nitrosyl hemoglobin and, upon oxygenation, the NO is transferred from the heme of β -subunits to thiol of β -cys93 to produce SNO-Hb. See specification at page 2, lines 12-16. More specifically, Applicants were the first to discover a method for preparing stable iron nitrosylated hemoglobin readily convertible to SNO-hemoglobin by reacting a low concentration of inorganic nitrite with deoxyhemoglobin (1:10 to 1:1000 mole ratios of nitrite to deoxy-hemoglobin) to form iron nitrosyl hemoglobin, which is a very desirable product because it is stable and generates a hemoglobin product capable of NO delivery (e.g., SNO-Hb) upon oxygenation.

These unexpected and superior properties of the claimed method are not taught or suggested by Stamler '006.

Applicants request reconsideration and withdrawal of the present rejections.

35 U.S.C. § 103

Claim 8 in part is rejected under 35 U.S.C. 103(a) as being unpatentable over Stamler (WO 96/30006) as applied to claims 3, 7, 9, 10, 12 above, and further in view of Remy *et al.* As stated above, Stamler '006 fails to teach or suggest all elements of the claims. At least, Stamler '006 fails to teach or suggest co-administering deoxygenated hemoglobin containing blood substitute and inorganic nitrite into the patient via infusion, wherein the inorganic nitrite is infused at a rate of 0.01 to 10 micromoles per minute, and the deoxygenated hemoglobin containing blood substitute is infused at a rate of 1 to 1000 cubic centimeters per hour. The secondary reference of Remy does not cure these deficiencies of Stamler '006, nor does the examiner rely on Remy to cure these deficiencies. According, the combination of Stamler '006 and Remy fail to teach or suggest all of the recited claim elements and thus cannot render claim 8 obvious. Withdrawal of this rejection is respectfully requested.

35 U.S.C. § 103

Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over WO 96/30006 as applied to claims 3, 7, 9, 10, 12 above, and further in view of US 4,820,269. As stated above, Stamler '006 fails to teach or suggest all elements of the claims. At least, Stamler '006 fails to teach or suggest co-administering deoxygenated hemoglobin containing blood substitute and inorganic nitrite into the patient via infusion, wherein the inorganic nitrite is infused at a rate of 0.01 to 10 micromoles per minute, and the deoxygenated hemoglobin containing blood substitute is infused at a rate of 1 to 1000 cubic centimeters per hour. The secondary reference of the '269 patent does not cure these deficiencies of Stamler '006, nor does the examiner rely on the '269 patent to cure these deficiencies. According, the combination of Stamler '006 and the '269 patent fail to teach or suggest all of the recited claim elements and thus cannot render claim 11 obvious. Withdrawal of this rejection is respectfully requested.

APPLICANTS: Stamler
SERIAL NUMBER: 10/538,404

Conclusion

Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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